Journal of Cosmetics, Dermatological Sciences and Applications, 2023, 13, 277-301
https://www.scirp.org/journal/jcdsa
ISSN Online: 2161-4512
ISSN Print: 2161-4105

# Exploring the Therapeutic Potential of Algae-Based Sheet Masks in Skincare: A Comprehensive Study of Cosmetological Benefits and Microbiome Balanced Interactions

**Ciska Janssens-Böcker, Karin Wiesweg, Claudia Doberenz**

MedSkin Solutions Dr. Suwelack, Billerbeck, Germany
Email: ciska.janssens-boecker@medskin-suwelack.com

**How to cite this paper:** Janssens-Böcker, C., Wiesweg, K. and Doberenz, C. (2023) Exploring the Therapeutic Potential of Algae-Based Sheet Masks in Skincare: A Comprehensive Study of Cosmetological Benefits and Microbiome Balanced Interactions. *Journal of Cosmetics, Dermatological Sciences and Applications*, **13**, 277-301.
https://doi.org/10.4236/jcdsa.2023.134023

**Received:** October 11, 2023
**Accepted:** December 9, 2023
**Published:** December 12, 2023



## Abstract

Research in dermatology is exploring bio-compatible materials like alginate and calcium sulphate for use in skincare products, such as facial sheet masks. Alginate (from seaweed) has wound healing and hydration properties, while calcium assists in maintaining skin balance and protection. In this study we explore the effects of an algae-calcium based sheet mask on skin. **Materials and Methods:** *In-vitro* studies investigated the purpose, process and effectiveness of an algae-calcium dry sheet mask (Algae Mask - MedSkin Solutions Dr. Suwelack). The mask is made from brown algae and undergoes a lyophilization and stabilization process to form its final structure. The mask's properties were tested using scanning electron microscopy (SEM) and its performance was assessed on human skin biopsies. Various tests were performed, such as measuring the release of ionic constituents and concentration of calcium ions, and the fibroblast cell-activating effect. Clinical evaluations and skin microbiome assessment were conducted on healthy volunteers to assess the mask's effect on the skin. **Results and Conclusion:** The study validates the benefits of macro algae-based sheet masks for skincare, with significant cosmetic effects and high tolerance due to its composition. Notably, the algae based mask releases substantial calcium ions, hydrates, minimizes skin roughness and wrinkles, regulates pH levels, and maintains skin microbiome diversity. It also decreases *Corynebacterium* species found on the skin, suggesting potential to modify skin microbiome.

## Keywords

Algae, Sheet Mask, Calcium, Skin Aging, Hydration, Microbiome, Skin pH

## 1. Introduction

Skin-related issues, such as acute dryness, environmental damage, and premature aging, are common problems experienced by many individuals worldwide. As science propels forward, we continue to look for novel treatments and prevention strategies with enhanced efficiency and biocompatibility. One emerging area of interest in the field of dermatology and skincare is the development of bio-compatible materials and their application in facial sheet masks. Among the most innovative of these materials are alginate and calcium [1] [2]. The potential health benefits, enhanced skin penetration, and the easy applicability of such masks make this study highly relevant.

Sheet masks are a type of facial treatment product designed to deliver intensive moisturization and active ingredients to the skin in a convenient, hygienic, single-use format. In its simplest form, a sheet mask is a face-shaped fabric that is soaked in a nutrition-packed solution, known as serum, which is designed to hydrate, soothe, and treat various skin conditions.

Alginates, naturally occurring polysaccharides isolated from brown seaweeds, have many medicinal properties including the ability to promote wound healing, anti-inflammatory effects, and antioxidant capacity [3] [4]. Because of their inherent ability to absorb water quickly, alginates can provide much-needed hydration to the skin, making them particularly useful in the formulation of sheet masks.

Algin, an anionic heteropolysaccharide extracted from natural brown algae, is composed of $\beta$-(1-4) linked D-mannuronic acid and $\alpha$-L-guluronic acid units [5]. Along its polymer chain, alginate has regions rich in sequential mannuronic acid units, guluronic acid units and regions in which both monomers are equally prevalent. Alginate can form strong hydrogels in the presence of divalent cations, such as $Ca^{2+}$, that interact with the carboxylic groups present in the alginate backbone to form ionic crosslinks [6] [7].

The inclusion of calcium sulphate in skincare therapies can attribute a note of innovation to the skincare industry, and this bioceramic has slowly been creating its own standing. Calcium ions play an integral part in our skin's physiology. They serve as key messengers in a range of cellular processes, including proliferation, differentiation, and barrier functions [8] [9] [10]. Calcium gradients exist within the outermost skin layer (the epidermis), with a primary function in regulating keratinocyte differentiation, the skin cells responsible for creating the protective barrier functionality and formation of the epidermal permeability barrier [10] [11] [12].

In the context of skincare treatments, calcium promotes enhanced skin barrier function, which is crucial in maintaining skin hydration and protection against harmful external elements. As evidenced in numerous research studies, variations in extracellular calcium concentrations can greatly affect skin barrier homeostasis [12] [13] [14] [15] [16].

Calcium sulphate is a bio-ceramic that holds immense potential in the realms

of drug delivery and skin health enhancement. This is a biocompatible material, renowned for its bioactivity, and osteoinductive properties [17] [18] [19]. Its potential application in transdermal ingredient delivery has given it a prominent position in recent research endeavors [19]. Transdermal drug or ingredient delivery systems aim to deliver drugs/ingredients through the skin to achieve therapeutic effects, making it a crucial aspect of skincare treatment [18].

The inclusion of such a material in skincare, especially in sheet masks, could improve product performance and effectiveness in delivering active ingredients to the skin.

This manuscript explores the effectiveness and potential dermatological benefits of a sheet mask composed of alginate and calcium sulphate.

## 2. Materials and Methods

### 2.1. Algae Mask

In the tests and evaluations in this study an algae-calcium based sheet mask was used, which is a waterless, preservative free, algae-calcium dry sheet mask (Algae Mask - [MedSkin Solutions Dr. Suwelack, Billerbeck, Germany]. The Algae Mask is processed in accordance with Good Manufacturing Practice according to Cosmetic Regulation (EG) 1223/2009. It is derived from brown algae (Lessonia trabeculata) that are purified, treated with a.o. sodium alginate and calcium sulphate and then lyophilized to form a biomatrix in the form of a facial sheet mask (INCI: Algin, cellulose gum, glycerin, calcium sulphate, caprylic/capric triglyceride, citric acid, sorbitol).

To determine the structure of the Algae Mask, scanning electron microscopy (SEM) was used at various magnifications. The effectiveness of the mask in soothing and managing irritated skin was assessed using an *in vitro* model system for skin. Human skin biopsies were acquired, and minor irritation was induced using a standardized cytokine protocol. The disturbed skin samples then received treatment with the Algae Mask, and skin specimens were gathered pre- and post-application. Scanning electron microscopy was utilized for investigating these samples and procuring side view images of the skin.

### 2.2. *In-Vitro* Tests

To determine whether the Algae Mask contains and releases calcium ions and their capability to penetrate skin and mimic the use of the mask on facial skin, two *in-vitro* tests were performed: the measurement of calcium ion release and the concentration of released calcium.

### 2.3. The Release of Ionic Constituents

The measurement of ionic release is executed within a Franz diffusion cell, where deionised water is utilized as the receiving medium. An Algae Mask segment is affixed to a skin substitute to mimic the placement as a sheet mask on skin. A dialysis membrane with a MWCO of 1 kDa, which had been previously

rinsed with RO water for three days, was utilized as a skin substitute. This rinsing process effectively removed any possible salts, ensuring that only very small dissolved particles could pass through the barrier, with the aim of blocking any other ingredients. A defined piece of the mask was applied on top of this substitute. The amount of water recommended when applying the mask was used in order to moisten this sample.

Beneath the substitute in the Franz cell, demineralised water was kept at a regulated temperature of 37 degrees Celsius and continuously agitated to replicate the actual blood flow beneath the skin. The decision to not use a real skin substitute, such as from nude mice, was due to the focus on analysing the amount of calcium that reaches and penetrates the skin during mask application, rather than the calcium that gets into the bloodstream.

At determined intervals, the water beneath the skin substitute in the Franz cell and the mask sample on top of it was entirely replaced. The samples were then mixed with a reagent from a Ca detection kit (Merck) and measurements were taken using the UV Vis. The quantity of released calcium was consequently calculated using the calibration series. The final step of the process involves tracking the ionic conductivity in the receiving medium over a span of 40 minutes. This is accomplished using a conductivity measuring instrument (WTW inoLab 750, Weilheim).

### 2.4. Determination of the Released Calcium Ion Concentration

The release profile of calcium ions was defined photometrically over a timeframe of 40 minutes, utilizing the technique of UV/VIS spectrometry (Perkin Elmer) at a wavelength of 565 nm. The release of calcium ions was measured using a Franz diffusion cell, with deionised water serving as the acceptor medium. A segment of the Algae Mask was affixed to a skin substitute to mimic the placement as a sheet mask on skin. The mask material was then dampened with deionised water, acting as the activating liquid. Finally, the concentration of the released calcium ions was measured using a photometric calcium assay.

### 2.5. Fibroblast Cell-Activating Effect

A XTT assay was applied to elucidate the potential stimulating effect of the Algae Mask's extract on fibroblast cells. By employing fibroblasts, which are common cells in connective tissue, this assay provides insights into how the mask influences cellular activity, an important factor considering the role of fibroblasts in wound healing and tissue regeneration. The metabolic activity of the fibroblast cells post-treatment with the mask extract is reflected by the intensity of the orange formazan dye produced. A higher color intensity indicates greater metabolic activity.

An extract of the Algae Mask was eluted with deionised water. The extract was dried in a drying cabinet at 60˚C and then absorbed with deionised water at a concentration of 50 mg/ml. The mask extract was further diluted to a tar-

get concentration in the range of 0.004 - 0.5% v/v in the cell medium (RPMI 1640, Gibco, Invitrogen). The ATCC, CCL NCTC L929 cell line (DSMZ, Brunswick, mouse connective tissue fibroblasts) was then employed for the cell culture. Subsequently, the cells were incubated at a temperature of 37˚C ± 1.5˚C and a $CO_2$ concentration of 5% ± 1.5% for a 24-hour duration. Following the incubation period, the XTT marker solution was introduced, which based on the cellular activity level, produces an orange dye known as Formazan. An optical density determination was made at a wavelength of 450 nm referenced against a wavelength of 690 nm, conducted via a microtiter plate reader (Versamax Molecular Devices). Seven sample measurements were taken from each concentration. For positive controls, test media were employed, supplemented with a sodium lauryl sulphate (SDS) solution at concentrations ranging from 250 to 3125 µl/ml.

## 2.6. Clinical Evaluation

In line with the stipulations of EU cosmetic Regulation no. 1223/2009, the study product was assessed for its safety before the clinical studies were conducted, thus alleviating the need for obtaining ethical approval. This regulation asserts that a cosmetic product should not be detrimental to human health under normal or logically anticipated conditions. All procedures conducted adhered to the ethical guidelines laid out in the Declaration of Helsinki. Participants of the study were provided sufficient information about the study and gave their consent to participate.

The study was executed by Dermatest GmbH (Münster, Germany). Before the first measurement and product application a wash-out phase of 10 days took place. Subjects underwent an acclimatization process for 45 minutes at a temperature of 22 degrees Celsius and 60% relative humidity.

Prior to initiating the application experiment, all test participants were subjected to a dermatological evaluation. Each participant's face was cleaned using Avène - Micellaire Cleansing Lotion. Following the cleaning, a specialist assessed the skin type and condition of each individual. The skin was then treated with Avène - Lotion douceur and Thermal Water Spray. Immediately after these steps, the first half of the mask was applied to the face and moisturized with water. The other half of the mask was then applied to the other side and moistened with a specific formulated liquid, Activation Liquid (INCI: aqua, butylene glycol, glycerin, pentylene glycol, lactobacillus fermente filtrate, sodium chloride, sodium hyaluronic). The mask was further shaped using fingertips or a flat spatula for an improved fit.

Following a 20-minute application period, the mask was carefully peeled off, starting from the bottom and rolling it upward.

Corneometer skin measurements were taken at three distinct locations within each corresponding test area, and an average of these readings was computed. The depth of wrinkles was examined before and after the period of application

by means of optical 3D measurement (PRIMOS, $R_z$ value). A dermatological assessment was done, specifically for scaling, erythema and dryness. Subject assessment criteria were collected with questionnaires.

In this experiment, the untreated skin adjacent to the test area was used as a control measurement zone. The recorded measurements were before the application, as well as 10 - 15 minutes and 120 minutes post-application. All measurements were carried out at least 10 - 12 hours after the participant's last application of any previously used product. The study panel comprised of 10 adult female participants with normal skin.

To qualify for inclusion, the subjects required healthy skin in the test area and had to be aged 40 years or older. Exclusion criteria included severe or chronic skin inflammation, serious internal or chronic diseases, and intake of drugs that could potentially interfere with skin reactions (like Glucocorticoids, antiallergics, topical immunomodulators, etc.).

Subjects who applied pharmaceutical products or skin care items containing active ingredients up to 7 - 10 days before testing were likewise excluded. The exclusion criteria also encompassed individuals with serious allergies or who had severe side effects after using cosmetic products, those who had sunbathed or used a tanning bed during the study period, known cancer patients, and individuals who were pregnant or lactating.

### 2.7. Clinical Microbiome Evaluation

This clinical investigation was designed to comparatively assess the diversity of skin microbiota before and after a 28-day application of an Algae Mask hydrated with Activator Liquid. Additional aims of this study were to investigate variations in skin microbiota diversity, skin pH, and transepidermal water loss (TEWL) between treated and untreated skin regions, as well as to observe alterations in skin pH and TEWL following a consecutive 28-day application of products compared to baseline values.

Twenty-eight healthy female participants, aged between 50 and 60 years with a baseline pH ≥ 5.5, participated in this study. Inclusion criteria required the participants to be post-menopausal, with at least one year having passed since their last menstrual cycle before the start of the investigation. Participants were also required to maintain their usual routines regarding exercise, skin hygiene, and dietary habits throughout the study period. Main exclusion factors included any present skin conditions, immunosuppression, or ongoing pharmacological treatment or antibiotics. Participants were asked not to apply any topical products (cosmetic or medical) to the area under evaluation (the forearms) either in the 24 hours leading up to the first measurement visit or throughout the 28-day investigation, including makeup and other skin products such as moisturizers and body lotions. The only exceptions were cleansing products and the investigational products. Participants were also asked not to swim during the investigation or to use hot tubs, steam baths, or saunas in the 48 hours immediately pre-

ceding the scheduled measurement visits.

The Algae Masks were applied every other day on the forearm, in the evenings, for a period of 28 days. The investigational product and the untreated skin site were randomly assigned to an outlined site of 3 × 3 cm on the volar part forearm with the help of a plastic marker.

Premoistened swabs were collected from each skin site of each subject before products' application (Day 0) and again 28 days after consecutive product applications (Day 28). Swabs for each subject were collected at the same time of day for each time-point of analysis. After collection, DNA was extracted to preserve the integrity of the sample, and Metagenomics NGS was used to assess the DNA samples (MiSeq platform - Illumina Inc. or Oxford Nanopore platform - Oxford Nanopore Technologies). After amplification, the quantity and integrity of the DNA were verified and normalized by agarose gel electrophoresis. The total number of copies of 16S rRNA was quantified and the results were expressed as the percentage of each species relative to the total number of all species present in the skin. The analysis included the global state of the skin microbiome, the individual analysis of the species *P. aeruginosa* and *C. acnes*, and the calculation of the Shannon's Diversity index.

Skin pH was measured in triplicate on each site using the equipment HALO pH/TEMP PROBE HI14142 (Hanna Instruments, Romania) before product application (Day 0) and 28 days after consecutive product applications (Day 28). TEWL was also measured on each site using a Tewameter® TM 300 (Courage+Khazaka electronic GmbH, Germany) before product application and 28 days after consecutive products' applications (Day 28).

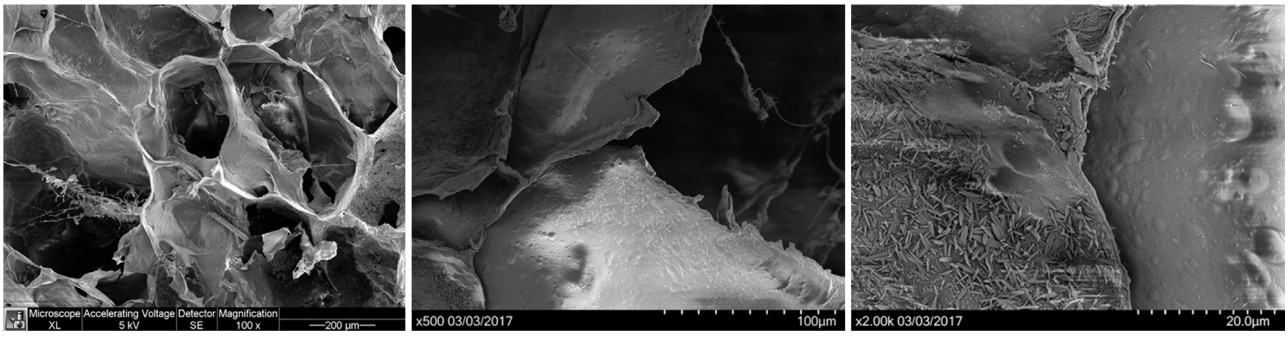
## 3. Results

### 3.1. Algae Mask

The Scanning Electron Microscopy (SEM) imaging shows that the mask consists of a biomatrix-ionic structure and predominantly comprises a 100% purified extract derived from algae and calcium, acting as a reservoir for essential minerals which is attributed to the lyophilization and stabilization process. The constitution of the bioactive component matrix in the Algae Mask bears a close resemblance to a natural sponge, complete with intricate branches, providing stable locations for ion adsorption. See **Figure 1**.

The Scanning Electron Microscopy (SEM) outcomes of the skin irritation specimens show a reduction in open spaces and gaps in the skin structures and a smoother skin surface after treatment with the mask. The skin shows a noticeable decrease in the dermal-epidermal junction (DEJ) gap, implying a potential enhancement in general skin health and stability (**Figure 2**).

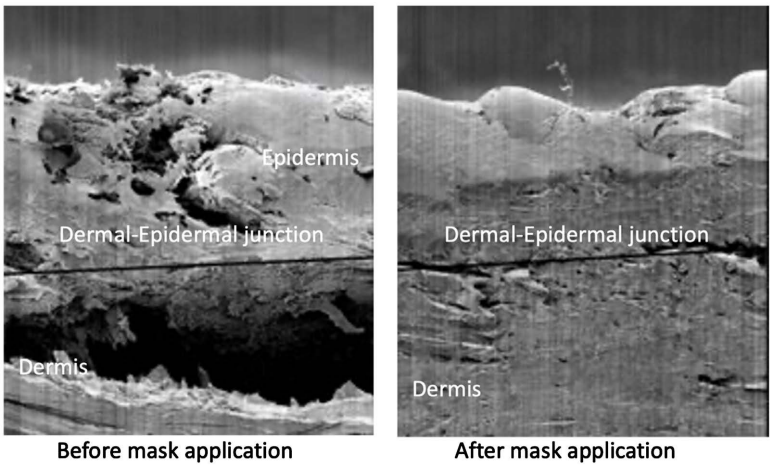
The reduction of open spaces and gaps in the human skin biopsies as shown in the SEM results suggest that the mask aided in alleviating the distress of the irritated skin explants. It also appeared to increasing moisture content and skin plumpness.




(a) (b) (c)

SEM images of Algae Mask at 100× magnification (a), 500× (b), 2000× (c), demonstrating the porous structure of the biomatrix consisting of crosslinked marine polysaccharides and calcium crystallites.

**Figure 1.** Scanning Electron Microscope (SEM) image (magnification 100× (a), 500× (b), 2000× (c)).




Before mask application After mask application

Side view SEM images of irritated human skin biopsies at 500× magnification, before and after mask application.

**Figure 2.** Scanning Electron Microscopy (SEM) image (500×).

### 3.2. The Release of Ionic Constituents

The conductivity, and thus the concentration of released ions, increased significantly during the period of measurement; at the end of the recommended period of mask application of 20 minutes, was approx. 8 mS/cm (**Figure 3**). These results indicate that calcium ions are released from the mask over time. After 20 minutes this release is slowed down. Initially, the minimal concentration of ions induced the infiltration of moisture into the skin cells through osmotic forces. Hypothetically, the retention of this introduced moisture could boost cell volume, resulting in a smoothing effect on lines and wrinkles. Notably, even after the application, the conductivity remained sufficiently low, thereby inhibiting the subsequent water loss from the cells induced by osmotic pressure and thus aiding in the preservation of skin's smoothness.

### 3.3. Determination of the Released Calcium Ion Concentration

The calcium concentration increased significantly initially during the 20-minute

treatment to 41.4 mg/l, then only slightly (to 52.4 mg/l) (Figure 4). In combination with the moisture absorption by the skin brought about by the osmotic effect, a low calcium concentration was initially provided so moisture could penetrate the deeper layers of the skin. Towards the end of the test, the concentration of the calcium ion was sufficiently high that an effective influence of the epidermal calcium gradient can occur [20] [21] [22]. In order to reproduce the conditions of young skin, there should only be a low-level influence of the calcium concentration in

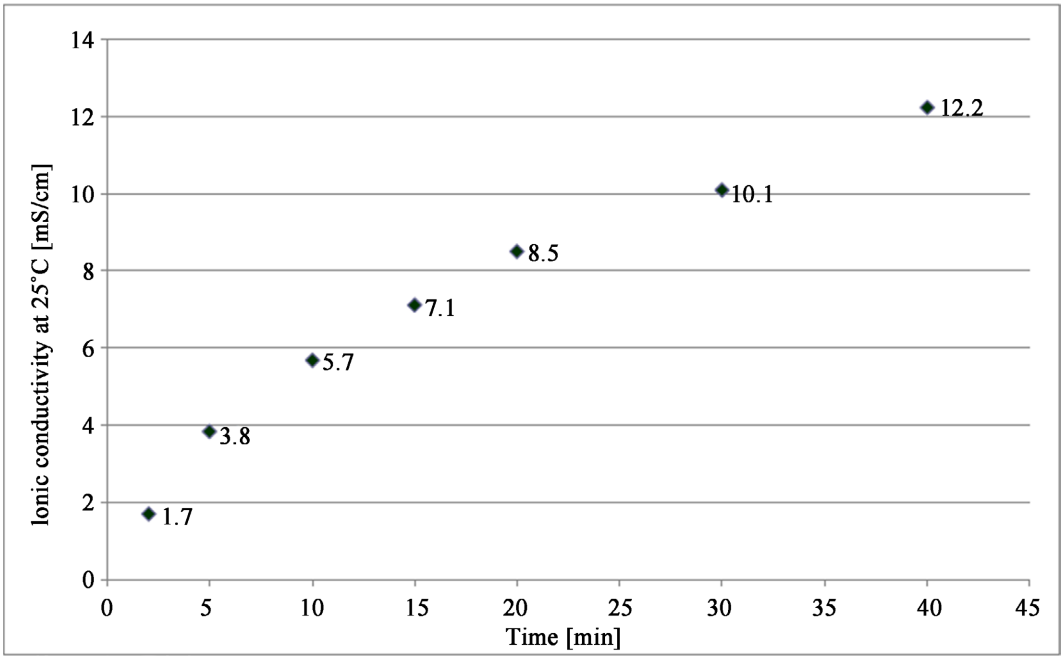



Figure 3. Calcium ion conductivity over time.

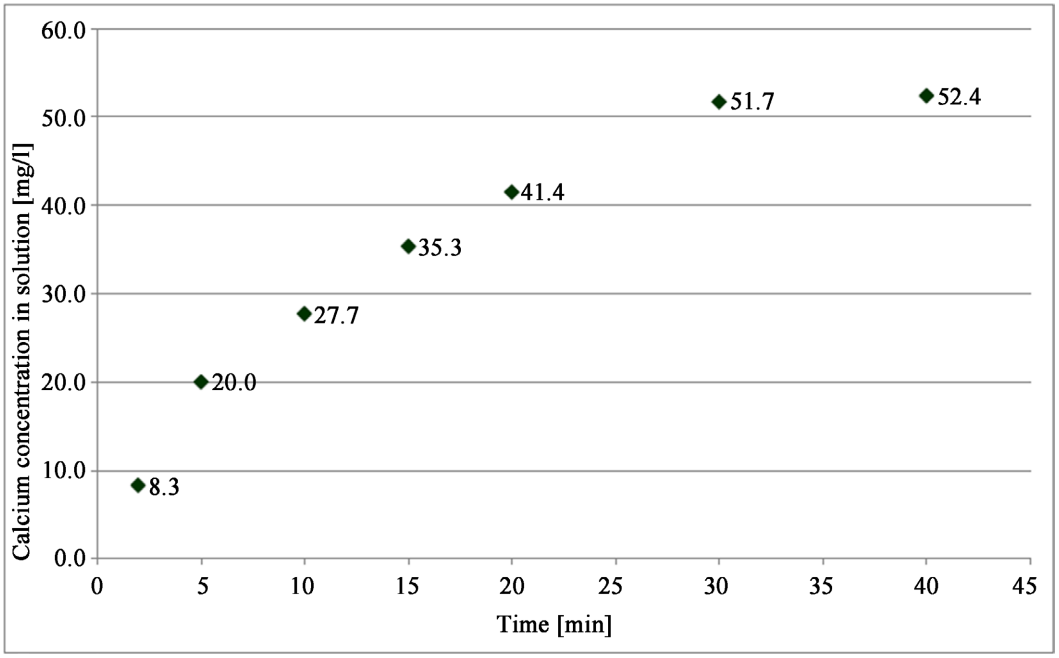



Figure 4. Calcium concentration over time.

the deeper layers, whereas the calcium concentration should be significantly increased close to the surface [21] [22] [23].

The results of this test indicate that calcium ions were discharged from the mask and effectively permeated through the skin substitute, a process indicating their potential to be absorbed and penetrate deeper skin layers. Noteworthy to highlight, during the opening phase of our *in-vitro* examination, there was a substantial rise in the calcium concentration. It augmented to 41.4 mg/l (corresponding to 0.9 mM), within a 20-minute treatment span. This increase reached an ample concentration, creating a viable environment for potentially enhancing differentiation of keratinocytes, replenishing drained calcium levels, and facilitating a more normalized calcium gradient.

### 3.4. Fibroblast Cell-Activating Effect

A colorimetric XTT assay is a widely recognized biochemical procedure conducted to measure cellular metabolic activity. The XTT assay was utilized to examine the possible stimulatory effect of the mask extract on fibroblast cells. The metabolic activity of the fibroblast cells post-treatment with the mask extract was reflected by the intensity of the orange formazan dye produced.

The mask extract (2.5 mg/ml) increased cellular activity significantly by 7% (mean value t-test) versus the untreated control cells (**Figure 5**).

This *in vitro* test therefore confirmed an increase in cellular activity resulting from the influence of the soluble constituents of the Algae Mask, implying that the mask may have the capacity to increase fibroblast activity.

### 3.5. Clinical Evaluation

All study participants were confirmed to have healthy skin in the test area at the onset, with no skin disorders detected. Throughout the application test, no

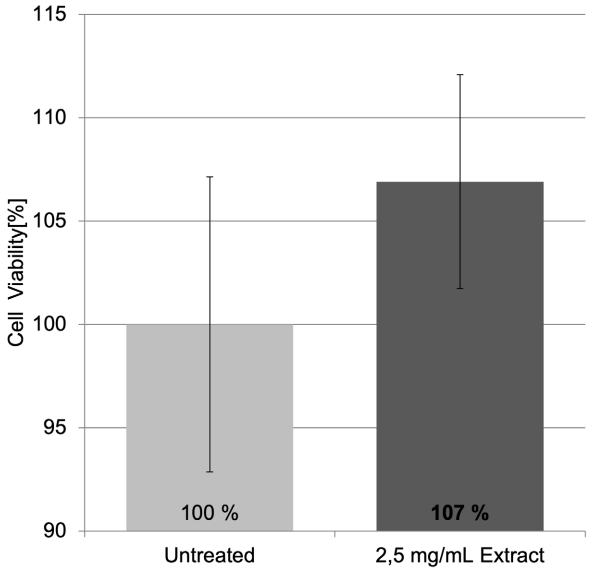



**Figure 5.** Cell viability of mouse skin fibroblast treated with an algae mask extract versus control.

issues relating to skin disorders were reported, and it was not necessary to interrupt the test or seek medical intervention.

At the conclusion of the application test, a final skin examination showed that 9 of the 10 participants had no evidence of developing any skin disorders in the test area. The sheet mask was well-tolerated and did not elicit any skin disorders. Only one participant presented skin redness on the left half of the face following product application on the side activated with water, but these minor irritations subsided without requiring dermatological treatment and were unlikely due to the mask.

The results from the corneometry measurements showed a significant increase in skin hydration when measured at different time intervals post application (Table 1 and Table 2, Figure 6).

When the mask was activated using water, we observed a 27% increase in skin hydration just 15 minutes following the application ($p < 0.01$). After a period of 2 hours, the hydration levels remained high, with a substantial increase by 26% ($p < 0.05$) suggesting the prolonged moisturizing effect of our mask.

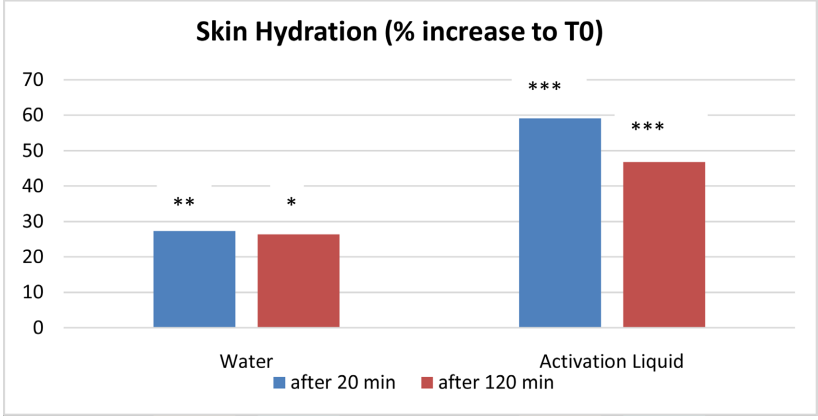
On the other hand, when using the Activation Liquid to trigger the mask, the hydration effect appeared to be more pronounced. After just 15 minutes of application, there was a significant increase in skin hydration by 59% ($p < 0.001$).

Table 1. Corneometry results after 15 - 20 minutes after mask removal.

| | before | 15 minutes after mask removal | Difference | Delta/Percent |
|---|---|---|---|---|
| **Control area** | | | | |
| average | 50.9 | 52.2 | 1.3 | 2.55 |
| Minimum | 41.5 | 41.4 | −3.7 | −7.91 |
| Maximum | 69.8 | 67.0 | 6.0 | 11.34 |
| Stand. dev. | 8.8 | 9.3 | 3.9 | 7.67 |
| Variance | 76.7 | 85.6 | 15.4 | 58.83 |
| **Activator: Water** | | | | |
| average | 53.3 | 69.2 | 15.9 | 29.83 |
| Minimum | 40.8 | 47.2 | 6.4 | 14.76 |
| Maximum | 66.9 | 83.3 | 27.2 | 54.73 |
| Stand.dev. | 8.3 | 10.6 | 7.1 | 14.81 |
| Variance | 68.7 | 113.3 | 50.1 | 219.42 |
| **Activator Liquid** | | | | |
| average | 51.3 | 81.1 | 29.8 | 58.09 |
| Minimum | 43.3 | 75.7 | 22.6 | 42.56 |
| Maximum | 57.3 | 86.9 | 35.0 | 80.83 |
| Stand.dev. | 4.6 | 3.9 | 3.3 | 11.16 |
| Variance | 21.5 | 15.4 | 10.8 | 124.61 |

**Table 2.** Corneometry results after 2 hours after mask removal.

| | before | 2 hours after mask removal | Difference | Delta/Percent |
|---|---|---|---|---|
| **Control area** | | | | |
| average | 50.9 | 47.9 | −3.0 | −5.89 |
| Minimum | 41.5 | 0.0 | −44.0 | −100.00 |
| Maximum | 69.8 | 69.2 | 5.3 | 11.56 |
| Stand. dev. | 8.8 | 19.1 | 14.8 | 33.22 |
| Variance | 76.7 | 365.4 | 217.8 | 1103.56 |
| **Activator: Water** | | | | |
| average | 53.3 | 64.2 | 10.9 | 20.45 |
| Minimum | 40.8 | 47.9 | 7.1 | 13.29 |
| Maximum | 66.9 | 78.2 | 15.2 | 26.17 |
| Stand.dev. | 8.3 | 10.0 | 2.7 | 4.11 |
| Variance | 68.7 | 99.7 | 7.2 | 16.91 |
| **Activator Liquid** | | | | |
| average | 51.3 | 74.9 | 23.6 | 46.00 |
| Minimum | 43.3 | 61.0 | 15.0 | 27.27 |
| Maximum | 57.3 | 87.7 | 38.4 | 77.89 |
| Stand.dev. | 4.6 | 9.2 | 7.7 | 15.52 |
| Variance | 21.5 | 85.5 | 59.6 | 240.74 |




Average increase in moisture after 15 - 20 minutes (T1) and two hours (T2 after mask removal). *p ≤ 0.05 **p ≤ 0.01 ***p ≤ 0.001.

**Figure 6.** Moisture increase after 15 - 20 minutes and 2 hours.

After 2 hours after application, the hydration level was still high with a significant growth by 47% ($p < 0.001$). Hence, the results from the corneometer readings strongly underline the potent hydrating properties of the mask.

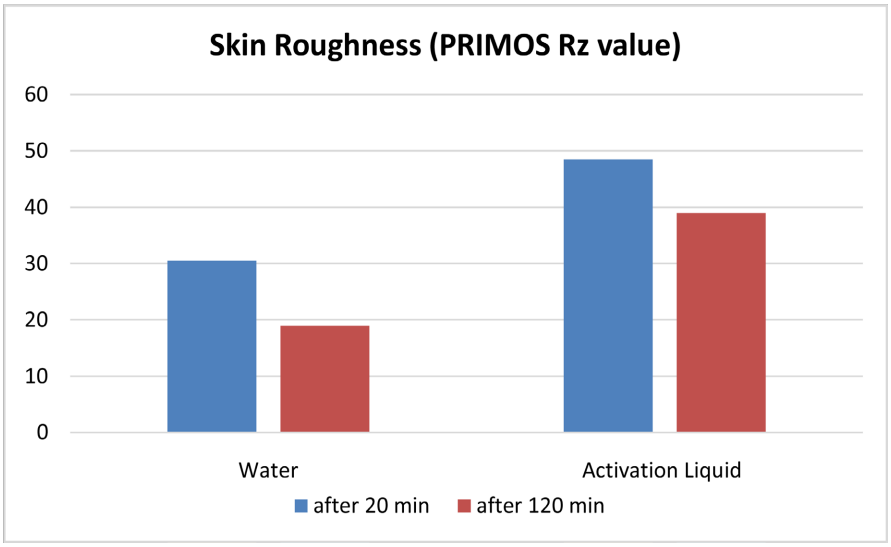
The PRIMOS results demonstrated a significant reduction in skin roughness

of 31% within the first 15 minutes of activation with water ($p < 0.001$), which remained after 2 hours with a significant reduction of 19% ($p < 0.05$). On the other hand, when activated with Activation Liquid, skin roughness saw a significant reduction of 49% within the first 15 minutes ($p < 0.001$). A substantial lasting effect was observed at the 2-hour mark, with skin roughness experiencing a significant increase of 39% ($p < 0.001$). (Table 3, Figure 7) These observations

**Table 3.** PRIMOS ($R_z$ value) after 15 minutes and 2 hours after mask removal.

| | $R_z$-Values µm | 15 minutes after mask removal $R_z$-Values µm | difference of $R_z$ µm | relative Change (%) |
|---|---|---|---|---|
| **Activator: Water** | | | | |
| average | 163.5 | 113.6 | −49.9 | −30.52 |
| Minimum | 140.8 | 84.4 | −97.2 | −53.11 |
| Maximum | 198.2 | 144.0 | −11.5 | −7.67 |
| Stand. dev. | 20.3 | 20.2 | 24.7 | 13.23 |
| Variance | 413.4 | 409.3 | 607.8 | 175.05 |
| **Activator: Water** | $R_z$-Values µm | 2 hours after mask removal $R_z$-Values µm | difference of $R_z$ µm | relative Change (%) |
| average | 163.5 | 132.5 | −31.0 | −18.96 |
| Minimum | 140.8 | 106.5 | −52.6 | −31.42 |
| Maximum | 198.2 | 163.4 | −5.2 | −3.47 |
| Stand. dev. | 20.3 | 17.3 | 16.1 | 8.74 |
| Variance | 413.4 | 298.1 | 258.2 | 76.38 |
| **Activator Liquid** | $R_z$-Values µm | 15 minutes after mask removal $R_z$-Values µm | difference of $R_z$ µm | relative Change (%) |
| average | 162.4 | 96.5 | −65.9 | −40.58 |
| Minimum | 144.9 | 64.6 | −93.9 | −59.24 |
| Maximum | 187.2 | 124.8 | −28.9 | −18.80 |
| Stand. dev. | 13.0 | 16.8 | 19.4 | 10.92 |
| Variance | 169.4 | 283.2 | 377.4 | 119.22 |
| **Activator Liquid** | $R_z$-Values µm | 2 hours after mask removal $R_z$-Values µm | difference of $R_z$ µm | relative Change (%) |
| average | 162.4 | 115.5 | −46.9 | −28.88 |
| Minimum | 144.9 | 89.9 | −63.9 | −38.89 |
| Maximum | 187.2 | 162.5 | −24.7 | −13.19 |
| Stand. dev. | 13.0 | 20.7 | 12.6 | 8.42 |
| Variance | 169.4 | 428.7 | 159.8 | 70.82 |

The $R_z$-DIN values in the test area are compared before and after in the relative change (with negative values indicating an improvement).




The $R_z$-DIN values in the test area are compared before and after in the relative change (with negative values indicating an improvement). *p ≤ 0.05 **p ≤ 0.01 ***p ≤ 0.001.

**Figure 7.** Reduction in skin roughness (PRIMOS, $R_z$ value) after 15 - 20 minutes and 2 hours after mask removal.

indicate activator liquid-dependent variations in PRIMOS outcomes.

### 3.6. Clinical Microbiome Evaluation

The mean results of the skin pH, evaluated before (t0) and after 28 days (t1) of products' application, are presented on **Table 4**. After 28 days of study performance, non-statistically significant ($p > 0.05$) differences were obtained for the untreated area_when compared to the skin pH results at the baseline.

The findings indicated a mean change of −2.03% in the skin pH values after 28 days of applying the mask every other day (t1), when compared to the initial baseline measurement (t0). Statistically significant variations ($p \leq 0.05$) were noticed between these two points in time. The results also showed a statistically significant difference ($p \leq 0.05$) when compared to areas that did not receive treatment. The data suggests that the Algae Mask has had a significant effect on skin pH levels, leading to a decrease in skin pH in 91.30% of the participants involved in the study. The maximum decrease noted was −7.02%.

**Table 5** presents the average measurements for transepidermal water loss (TEWL), taken before product application (baseline - t0) and following 28 days of use (t1). By comparing these two data points, the effect of the products on skin barrier integrity was evaluated. An improvement or stability in the TEWL scores hints at improved or maintained skin barrier function, respectively.

In the untreated area, statistically significant differences ($p \leq 0.05$) were observed after 28 days compared to the baseline TEWL values. Even though marginally elevated temperatures were recorded on day 28 compared to the baseline, the range of values registered at both time-points remained more or less consistent, signifying equal impact on all subjects. Consideration was given to the

**Table 4.** Skin pH evaluation.

| | Algae Mask | | Untreated area | |
|---|---|---|---|---|
| | t0<br>n = 23 | t1<br>n = 23 | t0<br>n = 23 | t1<br>n = 23 |
| Skin pH mean values | 5.82 | 5.70 | 5.77 | 5.75 |
| ± SD | 0.18 | 0.21 | 0.20 | 0.24 |
| | t1 − t0<br>n = 23 | | t1 − t0<br>n = 21 | |
| Mean diferences | −0.12 | | 0.02 | |
| ± SD | 0.11 | | 0.08 | |
| Mean differences (%) | −2.03% | | 0.27% | |
| ± SD (%) | 1.99% | | 1.45% | |
| No. of subjects with skin pH decrease | 21 | | 8 | |
| % of subjects with skin pH decrease | 91.30% | | 38.10% | |
| Maximum decrease (%) | −7.02% | | −3.15% | |
| Mean decrease among subjects with positive effects (%) | −2.24% | | −0.98% | |
| p value (before vs after product's application) | < 0.001* | | 0.507* | |
| p value (Investigational product vs untreated area) | < 0.001* | | – | |

*Paired t-test; **Wilcoxon test. Skin pH results obtained before (t0) and after 28 days (t1) of application of the Algae Mask and on the untreated area.

**Table 5.** TEWL results.

| | Algae Mask | | Untreated area | |
|---|---|---|---|---|
| | t0<br>n = 22 | t1<br>n = 22 | t0<br>n = 20 | t1<br>n = 20 |
| TEWL mean values | 6.07 | 9.28 | 6.14 | 8.52 |
| ± SD | 1.78 | 2.29 | 1.40 | 1.73 |
| | t1 - t0<br>n = 20 | | t1 - t0<br>n = 20 | |
| Mean differences | 2.85 | | 2.30 | |
| ± SD | 2.08 | | 1.81 | |
| Mean differences (%) | 53.70% | | 41.32% | |
| ± SD (%) | 47.76% | | 31.01% | |
| No. of subjects with TEWL decrease | 2 | | 3 | |
| % of subjects with TEWL decrease | 10.00% | | 15.00% | |
| Maximum decrease (%) | −11.60% | | −23.90% | |
| Mean decrease among subjects with positive effects (%) | −9.40% | | −9.89% | |
| p value (before vs after product's application) | <0.001* | | <0.001* | |
| p value (Investigational product vs untreated area) | 0.323* | | – | |

*Paired t-test; **Wilcoxon test. TEWL mean results obtained before (t0) and after 28 days (t1) of application of the Algae Mask and on the untreated area.

awareness that minor shifts in environmental conditions could influence the natural loss of skin moisture, which is prone to fluctuate over time. Consequently, these disparities were factored in while analyzing all results for comparison and considered as an ordinary skin response.

The Algae Mask noted a 53.70% mean variation in TEWL measurements after 28 days of bi-daily application (t1) in comparison to the baseline (t0). Between these two points, statistically significant differences ($p \leq 0.05$) were identified. Yet, when compared to the untreated skin area with a difference of 41.32%, these variations weren't statistically significant ($p > 0.05$). Based on these findings, it can be concluded that the TEWL decrease for the mask was no different from the natural fluctuations of the skin throughout the time.

Upon assessing the skin's microbiome at the genus level, results show that there are 4 dominant genera that remained dominant over time and were independent of treatment provided. (**Table 6**) Post a 28-day application period (t1) of the Algae Mask, it was observed that *Staphylococcus* was the dominant genus. It was followed by genera such as *Corynebacterium*, *Streptococcus*, *Cutibacterium*, *Enhydrobacter*, and *Acinetobacter*. These major genera were also consistently noticed both at the outset (t0), as well as in an untreated area after the mask's 28-day application period (t1). These findings underscore these genera as the key constituents of the skin microbiota.

A significant increase ($p \leq 0.05$) in the median Shannon's Diversity Index value was noted after 28 days of product's usage every other day (t1) when compared to the baseline (t0). However, this increase was not significantly different ($p > 0.05$) from the index results for the untreated area.

The relative abundance of the genus *Staphylococcus* decreased significantly ($p \leq 0.05$) over the two time-points. Yet, compared with results for the untreated area, this difference was not statistically significant ($p > 0.05$).

The differences in the relative abundance of the genus *Corynebacterium* and *Pseudomonas* between the two time-points weren't statistically significant ($p > 0.05$). Similarly, applying these results to the untreated area, no statistically significant difference ($p > 0.05$) was observed in *Pseudomonas*. However, a significant variation was observed in the relative abundance of *Corynebacterium* post 28 days of product's application (t1) every other day.

A statistically significant ($p \leq 0.05$) decrease of the relative abundance of the species *C. acnes* and *S. epidermidis* were observed between the two time-points. However, comparing with the results obtained for the untreated area, non-statistically significant differences ($p > 0.05$) were observed. For the species *S. aureus*, no significant differences ($p > 0.05$) were apparent between the two time-points or when compared to the untreated area (**Table 6**).

These findings suggests that the Algae Mask retained the skin microbiome diversity, causing no major shift in the overall skin microbiome, but did decrease the relative abundance of the genus *Corynebacterium* after 28 days of regimen (t1), which could potentially be linked to a decrease in skin pH.

**Table 6.** Microbiome results before and after 28 days.

| | Algae Mask | | Untreated Area | |
|---|---|---|---|---|
| | t0 n = 23 | t1 n = 23 | t0 n = 23 | t1 n = 23 |
| Shannon's Diversity index mean values | 5.40 | 5.79 | 5.31 | 5.69 |
| p value (before vs after product's application) | | 0.016* | | 0.023* |
| p value (Investigational product vs untreated area) | 0.577* | 0.551* | | |
| **Genus Relative Abundance %** | | | | |
| *Corynebacterium* | 10.71 | 8.63 | 9.89 | 11.80 |
| *Staphylococcus* | 19.45 | 15.12 | 19.28 | 15.25 |
| *Pseudomonas* | 1.44 | 1.85 | 2.12 | 2.05 |
| **p value (before vs after product's application)** | | | | |
| *Corynebacterium* | | 0.102* | | 0.249* |
| *Staphylococcus* | | 0.048* | | 0.146* |
| *Pseudomonas* | | 0.163* | | 0.810* |
| **p value (Investigational product vs untreated area)** | | | | |
| *Corynebacterium* | 0.905* | 0.018* | | |
| *Staphylococcus* | 0.973* | 0.900* | | |
| *Pseudomonas* | 0.194* | 0.978* | | |
| **Species Relative Abundance %** | | | | |
| *Cutibacterium acnes* | 8.95 | 6.03 | 7.84 | 4.97 |
| *Staphylococcus aureus* | 0.03 | 0.06 | 0.05 | 0.04 |
| *Staphylococcus epidermidis* | 7.93 | 5.17 | 7.76 | 5.77 |
| *Pseudomonas aeruginosa* | 0 | 0 | 0 | 0 |
| **p value (before vs after product's application)** | | | | |
| *Cutibacterium acnes* | | 0.008* | | 0.182* |
| *Staphylococcus aureus* | | 0.466* | | 0.940* |
| *Staphylococcus epidermidis* | | 0.012* | | 0.032* |
| *Pseudomonas aeruginosa* | | ** | | ** |
| **p value (Investigational product vs untreated area)** | | | | |
| *Cutibacterium acnes* | 0.737* | 0.924* | | |
| *Staphylococcus aureus* | 0.587* | 0.744* | | |
| *Staphylococcus epidermidis* | 0.996* | 0.756* | | |
| *Pseudomonas aeruginosa* | ** | ** | | |

Shannon's Diversity index, relative abundance of the genus *Corynebacterium*, *Staphylococcus* and *Pseudomonas* and relative abundance of the species *Cutibacterium acnes*, *Staphylococcus aureus*, *Staphylococcus epidermidis* and *Pseudomonas aeruginosa* obtained before (t0) and after 28 days (t1) of application of the Algae Mask. *ANOVA;**Not calculated.

## 5. Discussion

The results of this study contribute to the further knowledge on effectiveness and utilization of natural marine based ingredients in cosmetics and skincare. The utilization of macro algae in skin care, particularly in formulating masks, has gained traction in the field of cosmetology and dermatological science [24]. The shift from synthetic compounds to natural materials, including marine algae, has attracted significant interest from numerous researchers [24] [25]. This interest is due to the extensive pharmacological properties of algae and its minimal cytotoxicity effects on human cells. Polysaccharides constitute up to 76% of the dry weight of algae [26]. They are the predominant components of the cell wall in algae, involved in significant physiological processes [27] [28]. Macro algae are known for their abundant content of bioactive compounds, including carbohydrates, proteins, minerals, polyunsaturated fatty acids (PUFAs), fatty acids (FAs), antioxidants (e.g., polyphenols, tocopherols), and pigments such as carotenoids, suggesting potential utility in skin care applications [29] [30] [31] [32].

There is sparse literature on the clinical effects of algae based skincare and some initial evidence published suggests the effectiveness of algae sheet masks in skin care.

A placebo-controlled study under extensive sun exposure, demonstrated a notable decrease in skin glycation scores and histamine sensitivity due to the application of, a halophile microalga [33]. Important indicators of skin aging, such as the number of wrinkles and spots, also demonstrated considerable improvement compared to the placebo group.

We recently reported positive clinical results on skin hydration and anti-wrinkle effect of a marine algae based serum spray containing *fucus vesiculosous* extract and *ulva lactuca* extract [34].

In another study on blue lagoon (BL) algae, participants applied a serum containing BL algae or a control substance twice daily. Results showed that regular skin pigmentation, measured through colorimetry, did not significantly vary between serum- and control-treated skin. However, digital photography using cross-polarization and RBX technology showed a significant decrease in the number of pigment spots on the serum-treated side of the face compared to the control-treated side [35].

However, despite the perceived benefits of algae-based masks, comprehensive scientific evaluation of their therapeutic or aesthetic efficacy remains somewhat underrepresented in the published literature. The possible synergistic effects and effectiveness with other skincare ingredients, remain areas that need thorough exploration.

We conducted tests and clinical studies on a natural, brown algae based, porous biomatrix sheet mask containing calcium ions. The results of the study contribute to further enhancing the knowledge on the effectiveness of algae based ingredients, especially in a sheet mask format.

The mask in this study is composed of an ionic biomatrix predominately made up of a thoroughly purified extract, which is derived from algae and calcium without the need of preservatives and other sensitizing chemicals. Evidence has shown that applying facial sheet masks containing chemicals such as phenoxyethanol and methylparaben, may drive the exposure to hazardous chemicals to increase significantly [36]. Phenoxyethanol and methylparaben, frequently utilized preservatives in personal care products, have been reported to trigger contact dermatitis in consumers [37] [38].

The high purity, porosity and effectiveness of the Algae Mask are a result of the lyophilization process, typically known as freeze-drying. This configuration serves as an effective repository for vital mineral elements.

The structure and composition of ions within the active ingredient matrix have undergone scientific analysis. This included the evaluation of ion release upon activation of the biomatrix. The findings indicate that calcium ions are discharged in quantities significant for both biological and cosmetic effectiveness and are able to penetrate the skin. Owing to the process of osmosis, these ions are capable of targeting and infiltrating layers of the skin.

The current scientific literature does not provide a specific concentration of $Ca^{2+}$ (Calcium ions) that is effective for skin health. Calcium ions and their concentration gradient play an important role in skin function. It is involved in various processes such as skin barrier function, keratinocyte differentiation, skin regeneration, skin hydration, and protection against UV damage [12]. However, the optimal range of $Ca^{2+}$ concentration for these processes can vary significantly and is not well-defined. For example, one study found that increasing the calcium concentration to 1.2 mM in cultured human skin cells increased the production of keratinocyte differentiation markers [39]. In *in vitro* studies, an effective concentration of $Ca^{2+}$ used to promote keratinocyte differentiation is often between 0.6 and 1.4 mM and deemed by numerous studies as effective and safe for skin cell cultures. However, concentrations may vary depending on the specific study parameters and target outcomes [40] [41] [42].

An initial study showcased that the peak rate of keratinocyte proliferation was achieved approximately at 0.3 mM $Ca^{2+}$, however, it decreased at $Ca^{2+}$ concentrations lower than 0.1 mM under serum-free conditions [10] [39] [42] [43]. Notably, this correlates with the endogenous gradient of $Ca^{2+}$ in human epidermis.

A compromised skin barrier often exhibits a diminished or faulty calcium gradient in the epidermis [13]. This phenomenon is particularly apparent in the ageing process, where calcium signaling is hindered and the calcium gradient collapses [44]. Such a change in the skin's structure may help to explain why ageing skin experiences diminished barrier function and epidermal thinning. One potential therapeutic strategy could involve the topical application of calcium to replenish the skin's calcium stores, and this could benefit ageing skin.

To date, no comprehensive investigation into the skin absorption of cations under standardized conditions has been documented. Nonetheless, certain spe-

cific cations reach the deeper layers of the skin or even blood (in vivo) or recipient medium (*in vitro*). For example, a measurement of the flow of sodium ($Na^+$) and potassium ($K^+$) ions was observed in Franz cell experiments conducted by Tregear [45]. Additionally, a study examining the clinical use of Epsom Salt baths revealed a noticeable increase in blood levels of magnesium ions ($Mg^{2+}$) and sulphate ions ($SO_4^{2-}$) in patients who consistently take Epsom Salt baths [46], further demonstrating the skin's ability to absorb these ions. Conversely, *in vitro* studies have shown that trivalent cations, including chromium ($Cr^{3+}$), aluminium ($Al^{3+}$), indium ($In^{3+}$), and gallium ($Ga^{3+}$), collect in the superficial layers of the skin without penetrating deeper [47] [48] [49]. Laudanska *et al.* found human skin to be permeable to magnesium and calcium ions [50].

Our study differed in set up as we did not culture cells in a calcium rich medium for several hours, but applied the calcium ion containing mask to a skin substitute for only 20 minutes. We demonstrated that the calcium ions were released from the mask and diffused through the skin substitute, and are therefore capable of being absorbed by the skin and reach deeper skin layers.

During the initial phase in our *in-vitro* study, the calcium concentration showed a significant increase, elevating to 41.4 mg/l (equals 0.9 mM), at 20-minutes treatment, reaching a sufficient concentration for a potential increase in keratinocyte differentiation, replenishing calcium levels and establishing a more normal calcium gradient.

We also examined the effect of calcium on the cellular activity and demonstrated a significant increase in fibroblast cellular activity by application of the mask.

In addition, the porous freeze-dried matrix of natural algae fibers acts as a carrier and active in one and releases its active ingredients to the skin immediately after rehydrating the mask. The mask porosity together with the high-water absorption feature of the algae fiber both contribute to a strong hydration effect. The hydration and smoothing effect is even stronger with the rehydration with a specifically formulated activator liquid in comparison with mask rehydration with water. This is likely due to the combination of hyaluronic acid and a polymer matrix from cellulose derivatives, pullulan and red microalga, that strengthens and lengthens the effect from the Algae Mask.

In addition, the release of calcium from the mask can lead to an osmotic suction of water, which then leads to increased moisturization of the skin. Our data demonstrates that the mask plumps the skin, smooths and reduces fine lines and wrinkles and improves skin hydration and roughness. The substitution of calcium supports the epidermal keratinization process, helps to stabilize the epidermal barrier and gives the skin more resistance. Already one single application of the mask leads to a significant increase in hydration, a reduction of skin roughness and smoothing of wrinkles as demonstrated by our results.

The mask's acidic pH value of 3.4 aligns with the normal skin pH environment, encouraging the growth of indigenous microorganisms. This appears to be

advantageous for the skin microbiome, as evidenced by our findings. The mask has had a significant effect on skin pH levels, leading to a decrease in skin pH in 91.30% of the participants involved in the study, with a mean significant decrease of 2.03%. In addition, the TEWL remained similar to the untreated area, indicative of a normal barrier function. The data drawn from the microbiome research implies that after a 28-day regimen, the mask maintained the diversity of skin microbiome, without any significant alteration in its comprehensive composition. Nevertheless, there was a noticeable reduction in the relative profusion of the *Corynebacterium* genus. This species is a dominant skin microbe and is linked to inflammation and invasive infection under the right circumstances [51] [52]. Using the Algae Mask every other day for 28 days, lowers the abundance of this species, a phenomenon that might be correlated with a decrease in skin pH.

## 6. Conclusion

In conclusion, this study provides strong evidence for the beneficial effects of a macro algae-based mask for skincare. The key findings demonstrated significant cosmetic outcomes, along with excellent tolerance, attributed to the unique composition of the mask. The mask was able to release calcium ions in substantial amounts, contributing to its cosmetic effectiveness. Also, due to its natural and purified algae extract and calcium ions, the mask evokes strong hydration effect, reduces skin roughness, smoothes wrinkles and even influences skin pH levels. An interesting note from these findings is the Algae Mask's potential to maintain the diversity of skin microbiome without causing any major alterations, a beneficial characteristic for healthy skin. Using the mask lowers the abundance of the *Corynebacterium* species, indicating the potential to shift skin microbiome species. Therefore, this research results endorse the further use and exploration of macro algae in the field of cosmetology and skincare.

## Acknowledgements

We thank Dr. Mathias Mörgelin for providing the SEM images and results. The authors would like to acknowledge and extend their appreciation to all the individuals from MedSkin Solutions who contributed to the research projects.

## Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

## References

[1] Deng, Y., Yang, N., Okoro, O., Shavandi, A. and Nie, L. (2021) Alginate-Based Composite and Its Biomedical Applications. In: Deniz, I., Imamoglu, E. and Keskin-Gundogdu, T., Eds., *Properties and Applications of Alginates*, InTechOpen, London. https://doi.org/10.5772/intechopen.99494

[2] Adamiak, K. and Sionkowska, A. (2023) State of Innovation in Alginate-Based Ma-

terials. *Marine Drugs*, **21**, Article No. 353. https://doi.org/10.3390/md21060353

[3] Ahmad Raus, R., Wan Nawawi, W.M.F. and Nasaruddin, R.R. (2021) Alginate and Alginate Composites for Biomedical Applications. *Asian Journal of Pharmaceutical Sciences*, **16**, 280-306. https://doi.org/10.1016/j.ajps.2020.10.001

[4] Zdiri, K., Cayla, A., Elamri, A., Erard, A. and Salaun, F. (2022) Alginate-Based Bio-Composites and Their Potential Applications. *Journal of Functional Biomaterials*, **13**, Article No. 117. https://doi.org/10.3390/jfb13030117

[5] Kuznetsova, T., Andrukov, B., Besednova, N., Zaporozhets, T. and Kalinin, A. (2020) Marine Algae Polysaccharides as Basis for Wound Dressings, Drug Delivery, and Tissue Engineering: A Review. *Journal of Marine Science and Engineering*, **8**, Article No. 481. https://doi.org/10.3390/jmse8070481

[6] Malektaj, H., Drozdov, A.D. and de Claville Christiansen, J. (2023) Mechanical Properties of Alginate Hydrogels Cross-Linked with Multivalent Cations. *Polymers*, **15**, Article No. 3012. https://doi.org/10.3390/polym15143012

[7] Zhang, X., Wang, X., Fan, W., Liu, Y., Wang, Q. and Weng, L. (2022) Fabrication, Property and Application of Calcium Alginate Fiber: A Review. *Polymers*, **14**, Article No. 3227. https://doi.org/10.3390/polym14153227

[8] Wang, M., Sun, Y., Li, L., Wu, P., Dkw, O. and Shi, H. (2021) Calcium Channels: Noteworthy Regulators and Therapeutic Targets in Dermatological Diseases. *Frontiers in Pharmacology*, **12**, Article ID: 702264. https://doi.org/10.3389/fphar.2021.702264

[9] Tu, C.L., Oda, Y., Komuves, L. and Bikle, D.D. (2004) The Role of the Calcium-Sensing Receptor in Epidermal Differentiation. *Cell Calcium*, **35**, 265-273. https://doi.org/10.1016/j.ceca.2003.10.019

[10] Bikle, D.D., Xie, Z. and Tu, C.L. (2012) Calcium Regulation of Keratinocyte Differentiation. *Expert Review of Endocrinology & Metabolism*, **7**, 461-472. https://doi.org/10.1586/eem.12.34

[11] Subramaniam, T., Fauzi, M.B., Lokanathan, Y. and Law, J.X. (2021) The Role of Calcium in Wound Healing. *International Journal of Molecular Sciences*, **22**, Article No. 6486. https://doi.org/10.3390/ijms22126486

[12] Lee, S.E. and Lee, S.H. (2018) Skin Barrier and Calcium. *Annals of Dermatology*, **30**, 265-275. https://doi.org/10.5021/ad.2018.30.3.265

[13] Behne, M.J., Sanchez, S., Barry, N.P., Kirschner, N., Meyer, W., Mauro, T.M., Moll, I. and Gratton, E. (2011) Major Translocation of Calcium upon Epidermal Barrier Insult: Imaging and Quantification via FLIM/Fourier Vector Analysis. *Archives of Dermatological Research*, **303**, 103-115. https://doi.org/10.1007/s00403-010-1113-9

[14] Lee, S.H., Elias, P.M., Feingold, K.R. and Mauro, T. (1994) A Role for Ions in Barrier Recovery after Acute Perturbation. *Journal of Investigative Dermatology*, **102**, 976-979. https://doi.org/10.1111/1523-1747.ep12384225

[15] Menon, G.K., Elias, P.M., Lee, S.H. and Feingold, K.R. (1992) Localization of Calcium in Murine Epidermis Following Disruption and Repair of the Permeability Barrier. *Cell and Tissue Research*, **270**, 503-512. https://doi.org/10.1007/BF00645052

[16] Elias, P., Ahn, S., Brown, B., Crumrine, D. and Feingold, K.R. (2002) Origin of the Epidermal Calcium Gradient: Regulation by Barrier Status and Role of Active vs Passive Mechanisms. *Journal of Investigative Dermatology*, **119**, 1269-1274. https://doi.org/10.1046/j.1523-1747.2002.19622.x

[17] Dorozhkin, S.V. (2010) Bioceramics of Calcium Orthophosphates. *Biomaterials*, **31**,

1465-1485. https://doi.org/10.1016/j.biomaterials.2009.11.050

[18] Benson, H.A. (2005) Transdermal Drug Delivery: Penetration Enhancement Techniques. *Current Drug Delivery*, **2**, 23-33. https://doi.org/10.2174/1567201052772915

[19] Ene, R., Nica, M., Ene, D., Cursaru, A. and Cirstoiu, C. (2021) Review of Calcium-Sulphate-Based Ceramics and Synthetic Bone Substitutes Used for Antibiotic Delivery in PJI and Osteomyelitis Treatment. *EFORT Open Reviews*, **6**, 297-304. https://doi.org/10.1302/2058-5241.6.200083

[20] Tsutsumi, M., Denda, S., Inoue, K., Ikeyama, K. and Denda, M. (2009) Calcium Ion Gradients and Dynamics in Cultured Skin Slices of Rat Hindpaw in Response to Stimulation with ATP. *Journal of Investigative Dermatology*, **129**, 584-589. https://doi.org/10.1038/jid.2008.299

[21] Elias, P.M., Ahn, S. K., Denda, M., Brown, B.E., Crumrine, D., Kimutai, L.K., Kömüves, L., Lee, S.H. and Feingold, K.R. (2002) Modulations in Epidermal Calcium Regulate the Expression of Differentiation-Specific Markers. *Journal of Investigative Dermatology*, **119**, 1128-1136. https://doi.org/10.1046/j.1523-1747.2002.19512.x

[22] Mauro, T., Bench, G., Sidderas-Haddad, E., Feingold, K., Elias, P. and Cullander, C. (1998) Acute Barrier Perturbation Abolishes the $Ca^{2+}$ and $K^{+}$ Gradients in Murine Epidermis: Quantitative Measurement Using PIXE. *Journal of Investigative Dermatology*, **111**, 1198-1201. https://doi.org/10.1046/j.1523-1747.1998.00421.x

[23] Menon, G.K., Elias, P.M. and Feingold, K.R. (1994) Integrity of the Permeability Barrier Is Crucial for Maintenance of the Epidermal Calcium Gradient. *British Journal of Dermatology*, **130**, 139-147. https://doi.org/10.1111/j.1365-2133.1994.tb02892.x

[24] Thiyagarasaiyar, K., Goh, B.H., Jeon, Y.J. and Yow, Y.Y. (2020) Algae Metabolites in Cosmeceutical: An Overview of Current Applications and Challenges. *Marine Drugs*, **18**, Article No. 323. https://doi.org/10.3390/md18060323

[25] Álvarez-Gómez, F., Korbee, N., Casas-Arrojo, V., Abdala-Díaz, R.T. and Figueroa, F.L. (2019) UV Photoprotection, Cytotoxicity and Immunology Capacity of Red Algae Extracts. *Molecules*, **24**, Article No. 341. https://doi.org/10.3390/molecules24020341

[26] Saadaoui, I., Rasheed, R., Abdulrahman, N., Bounnit, T., Cherif, M., Al Jabri, H. and Mraiche, F. (2020) Algae-Derived Bioactive Compounds with Anti-Lung Cancer Potential. *Marine Drugs*, **18**, Article No. 197. https://doi.org/10.3390/md18040197

[27] Gupta, S. and Abu-Ghannam, N. (2011) Recent Developments in the Application of Sea-Weeds or Seaweed Extracts as a Means for Enhancing the Safety and Quality Attributes of Foods. *Innovative Food Science & Emerging Technologies*, **12**, 600-609. https://doi.org/10.1016/j.ifset.2011.07.004

[28] Cherry, P., O'Hara, C., Magee, P.J., McSorley, E.M. and Allsopp, P.J. (2019) Risks and Benefits of Consuming Edible Seaweeds. *Nutrition Reviews*, **77**, 307-329. https://doi.org/10.1093/nutrit/nuy066

[29] Menaa, F., Wijesinghe, U., Thiripuranathar, G., Althobaiti, N.A., Albalawi, A.E., Khan, B.A. and Menaa, B. (2021) Marine Algae-Derived Bioactive Compounds: A New Wave of Nanodrugs? *Marine Drugs*, **19**, Article No. 484. https://doi.org/10.3390/md19090484

[30] Khanna, P., Kaur, A. and Goyal, D. (2019) Algae-Based Metallic Nanoparticles: Synthesis, Characterization and Applications. *Journal of Microbiological Methods*, **163**, Article ID: 105656. https://doi.org/10.1016/j.mimet.2019.105656

[31] Menaa, F., Wijesinghe, P.A.U.I., Thiripuranathar, G., Uzair, B., Iqbal, H., Khan,

B.A. and Menaa, B. (2020) Ecological and Industrial Implications of Dynamic Seaweed-Associated Microbiota Interactions. *Marine Drugs*, **18**, Article No. 641. https://doi.org/10.3390/md18120641

[32] Hamid, N., Ma, Q., Boulom, S., Liu, T., Zheng, Z., Balbas, J. and Robertson, J. (2015) Seaweed Minor Constituents. In: Tiwari, B.K. and Troy, D.J., Eds., *Seaweed Sustainability*, Elsevier, Amsterdam, 193-242. https://doi.org/10.1016/B978-0-12-418697-2.00008-8

[33] Havas, F., Krispin, S., Cohen, M., Loing, E., Farge, M., Suere, T. and Attia-Vigneau, J. (2022) A *Dunaliella salina* Extract Counteracts Skin Aging under Intense Solar Irradiation Thanks to Its Antiglycation and Anti-Inflammatory Properties. *Marine Drugs*, **20**, Article No. 104. https://doi.org/10.3390/md20020104

[34] Janssens-Böcker, C., Wiesweg, K. and Doberenz, C. (2023) The Tolerability and Effectiveness of Marine-Based Ingredients in Cosmetics: A Split-Face Clinical Study of a Serum Spray Containing *Fucus vesiculosus* Extract, *Ulva lactuca* Extract, and Ectoin. *Cosmetics*, **10**, Article No. 93. https://doi.org/10.3390/cosmetics10030093

[35] Grether-Beck, S., Marini, A., Jaenicke, T., Brenden, H., Felsner, I., Aue, N., Brynjolfsdottir, A. and Krutmann, J. (2022) Blue Lagoon Algae Improve Uneven Skin Pigmentation: Results from *in Vitro* Studies and from a Monocentric, Randomized, Double-Blind, Vehicle-Controlled, Split-Face Study. *Skin Pharmacology and Physiology*, **35**, 77-86. https://doi.org/10.1159/000518781

[36] Zhou, L., Chen, J., Chen, T., Chang, H., Cui, W., She, Y., Li, Z., Tang, W., Yuan, D., Chen, Z. and Su, J. (2022) Investigation of Actual Exposure to Facial Sheet Mask Preceding Its Risk Assessment. *Scientific Reports*, **12**, Article No. 1215. https://doi.org/10.1038/s41598-022-05351-3

[37] Cheng, S., Leow, Y.H., Goh, C.L. and Goon, A. (2014) Contact Sensitivity to Preservatives in Singapore: Frequency of Sensitization to 11 Common Preservatives 2006-2011. *Dermatitis*, **25**, 77-82. https://doi.org/10.1097/DER.0000000000000031

[38] Beene, K.M., Scheman, A., Severson, D. and Reeder, M.J. (2017) Prevalence of Preservatives across All Product Types in the Contact Allergen Management Program. *Dermatitis*, **28**, 81-87. https://doi.org/10.1097/DER.0000000000000259

[39] Hennings, H., Michael, D., Cheng, C., Steinert, P., Holbrook, K. and Yuspa, S.H. (1980) Calcium Regulation of Growth and Differentiation of Mouse Epidermal Cells in Culture. *Cell*, **19**, 245-254. https://doi.org/10.1016/0092-8674(80)90406-7

[40] Huang, Y.-C., Wang, T.-W., Sun, J.-S. and Lin, F.-H. (2006) Effect of Calcium Ion Concentration on Keratinocyte Behaviors in the Defined Media. *Biomedical Engineering*: *Applications*, *Basis and Communications*, **18**, 37-41. https://doi.org/10.4015/S1016237206000087

[41] Borowiec, A.-S., Delcourt, P., Dewailly, E. and Bidaux, G. (2013) Optimal Differentiation of *in Vitro* Keratinocytes Requires Multifactorial External Control. *PLOS ONE*, **8**, e77507. https://doi.org/10.1371/journal.pone.0077507

[42] Boyce, S.T. and Ham, R.G. (1983) Calcium-Regulated Differentiation of Normal Human Epidermal Keratinocytes in Chemically Defined Clonal Culture and Serum-Free Serial Culture. *Journal of Investigative Dermatology*, **81**, 33s-40s. https://doi.org/10.1111/1523-1747.ep12540422

[43] Xie, Z., Singleton, P.A., Bourguignon, L.Y. and Bikle, D.D. (2005) Calcium-Induced Human Keratinocyte Differentiation Requires src- and fyn-Mediated Phosphatidylinositol 3-Kinase-Dependent Activation of Phospholipase C-gamma1. *Molecular Biology of the Cell*, **16**, 3236-3246. https://doi.org/10.1091/mbc.e05-02-0109

[44] Streubel, M.K., Neuhofer, C., Bischof, J., Steinbacher, P., Russe, E., Wechselberger,

G., Richter, K. and Rinnerthaler, M. (2018) From Mice to Men: An Evolutionary Conserved Breakdown of the Epidermal Calcium Gradient and Its Impact on the Cornified Envelope. *Cosmetics*, **5**, Article No. 35. https://doi.org/10.3390/cosmetics5020035

[45] Tregear, R.T. (1966) The Permeability of Mammalian Skin to Ions. *Journal of Investigative Dermatology*, **46**, 16-23. https://doi.org/10.1038/jid.1966.4

[46] Waring, R. (2014) Report on Magnesium Sulfate across the Skin. School of Biosciences, University of Birmingham, Birmingham.

[47] Pineau, A., Guillard, O., Favreau, F., Marrauld, A. and Fauconneau, B. (2012) *In Vitro* Study of Percutaneous Absorption of Aluminum from Antiperspirants through Human Skin in the Franz™ Diffusion Cell. *Journal of Inorganic Biochemistry*, **110**, 21-26. https://doi.org/10.1016/j.jinorgbio.2012.02.013

[48] Wahlberg, J.E. (1965) Percutaneous Absorption of Sodium Chromate (51Cr), Cobaltous (58Co), and Mercuric (203Hg) Chlorides through Excised Human and Guinea Pig Skin. *Acta Dermato-Venereologica*, **45**, 415-426.

[49] Bolzinger, M.A., Bolot, C., Galy, G., Chabanel, A., Pelletier, J. and Briançon, S. (2010) Skin Contamination by Radiopharmaceuticals and Decontamination Strategies. *International Journal of Pharmaceutics*, **402**, 44-49. https://doi.org/10.1016/j.ijpharm.2010.09.027

[50] Laudańska, H., Lemancewicz, A., Kretowska, M., Reduta, T. and Laudański, T. (2002) Permeability of Human Skin to Selected Anions and Cations—*In Vitro* Studies. *Research Communications in Molecular Pathology and Pharmacology*, **112**, 16-26.

[51] Ridaura, V.K., Bouladoux, N., Claesen, J., Chen, Y.E., Byrd, A.L., Constantinides, M.G., Merrill, E.D., Tamoutounour, S., Fischbach, M.A. and Belkaid, Y. (2018) Contextual Control of Skin Immunity and Inflammation by Corynebacterium. *Journal of Experimental Medicine*, **215**, 785-799. https://doi.org/10.1084/jem.20171079

[52] JDC, SLK and GJD-H (2009) Ralph D. Feigin, M.D.: April 3, 1938-August 14, 2008. In: Feigin, R.D., *et al.*, Eds., *Feigin and Cherry's Textbook of Pediatric Infectious Diseases*, 6th Edition, W.B. Saunders, Philadelphia, 5. https://doi.org/10.1016/B978-1-4160-4044-6.50002-9